*REMARKS/ARGUMENTS*

*The Pending Claims*

Claims 24, 29, and 30 are pending and directed to methods of preparing a creatine amidinohydrolase.

*Amendments to the Claims*

The claims have been amended to point out more particularly and claim more distinctly the invention. Specifically, claim 30 has been amended to recite "creatine" in place of "substrate" as supported by the specification at column 9, lines 14-39. No new matter has been added by way of this amendment. The precise changes to claim 30 are recited in Exhibit A.

*Summary of the Office Action*

The Office has objected to the specification because the Substitute Sequence Listing that corrects a typographical error in SEQ ID NO: 2 allegedly introduces new matter. The Office has rejected claim 30 under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description. Reconsideration is hereby requested.

*Discussion of Objection to the Specification*

The Office has objected to the specification because the Substitute Sequence Listing that was submitted with the previous Response to Office Action dated April 18, 2005 allegedly introduced new matter. The Office contends that applicants did not provide evidence that that nucleotide sequence contained an error other than that of attorney argument.

Applicants herewith submit a Rule 132 Declaration of Atsushi Sogabe, who is a co-inventor of the subject matter of the application. The Rule 132 declaration identifies the typographical error in the nucleotide sequence of SEQ ID NO: 2. Specifically, nucleotide residue 435 of SEQ ID NO: 2 should be guanine (G), and not cysteine (C). Thus, the codon at nucleotide residues 433-435 should be GAG, and not GAC. The deduced amino acid sequence of SEQ ID NO: 1 (which also appears in SEQ ID NO: 2) correctly sets forth the amino acid corresponding to the codon at nucleotide residues 433-435 as glutamine (Glu).

Moreover, the Rule 132 declaration confirms that the correct nucleotide sequence that encodes SEQ ID NO: 1 could be readily sequenced by one of ordinary skill in the art as of 1996 (the earliest priority date of the application) from the source material of the amidinohydrolase gene derived from *Alcaligenes faecalis* TE3581, which has deposit accession number FERM P-14237 (see, e.g., column 4, lines 7-10, of the application).

Thus, one of ordinary skill in the art, reading the specification of the present application as of its earliest priority date, would have appreciated the typographical error and understood the proper correction.

Since the Substitute Sequence Listing merely corrects a typographical error, the correct information for which could be readily obtained by an ordinarily skilled artisan based on the teachings in the specification and what was known in the art at the relevant time, no new matter was added by way of the Substitute Sequence Listing. Accordingly, Applicants respectfully request that the objection to the specification be withdrawn.

*Discussion of the Written Description Rejection*

The Office has rejected claim 30 for allegedly lacking written description. Specifically, the Office contends that the specification only has support for one substrate (creatine) and one set of concentrations (a given concentration and 1/10 thereof). Applicants traverse the rejection for the following reasons.

Claim 30, as amended, replaces the term "substrate" with "creatine" as suggested by the Office. Regarding the sets of concentrations, the Km value is determined by calculation based on an appropriate method, such as a Lineweaver-Burk plot, using the experimental data obtained by tests using several different substrate (i.e., creatine) concentrations. In the method described in Example 3 of the application, two concentrations (1 and 1/10) were employed for the convenience of screening and ease of calculation. One of ordinary skill in the art would readily understand that the determination of the Km value can be made using substrates at any two different concentration levels and that Applicants merely utilized a given concentration and 1/10 thereof as illustrative of the general technique.

For example, the enclosed reference (Segel et al., Biochemical Calculations: How to Solve Mathematical Problems in General Biochemistry, 2nd Ed., John Wiley & Sons, Inc., New York, 1979; English translation) describes the reaction mechanism for an enzyme-catalyzed conversion of a substrate (such as creatine) into a product. As indicated by the boxed sections of the translated document on pages 1 and 2, the same Km value will be obtained regardless of the concentration levels of substrate in the test (as long as the enzyme follows the formula of Herni-Michaelis-Menten, which assumes rapid equilibrium). Accordingly, one of ordinary skill in the art would appreciate that the method of the invention using a substrate (e.g., creatine) at any two concentration levels for the determination of Km is appropriate and was in the possession of the Applicants as evidenced by the information set forth in the present application.

For these reasons, the Applicants believe that the subject matter of the claims is adequately supported by the specification, such that the written description rejection should be withdrawn.

*Conclusion*

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

John Kilyk, Jr., Reg. No. 30,763
LEYDIG, VOIT & MAYER, LTD.
Two Prudential Plaza, Suite 4900
180 North Stetson Avenue
Chicago, Illinois 60601-6780
(312) 616-5600 (telephone)
(312) 616-5700 (facsimile)

Date: October 6, 2005

**EXHIBIT A - Additional Claim Amendments**
**(deletions indicated by brackets and additions underlined)**

The previously described claims were additionally amended as follows:

30. A method of preparing a creatine amidinohydrolase comprising:

(i) selecting (a) a nucleic acid sequence of SEQ ID NO: 2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 1 to provide a source nucleic acid sequence,

(ii) mutating the source nucleic acid sequence to provide mutant nucleic acid sequences that encode mutant creatine amidinohydrolases,

(iii) selecting a mutant nucleic acid sequence that encodes a creatine amidinohydrolase which has a reduced Km value as compared to the Km value of creatine amidinohydrolase encoded by the source nucleic acid sequence by:

(A) determining a first activity of creatine amidinohydrolase encoded by the source nucleic acid sequence with a first concentration of [a substrate] creatine and a second activity of creatine amidinohydrolase encoded by the source nucleic acid sequence with a second concentration of [the substrate] creatine, wherein the second concentration of [the substrate] creatine is less than the first concentration of [the substrate] creatine,

(B) determining a first activity of the mutant creatine amidinohydrolase with the first concentration of [the substrate] creatine and a second activity of the mutant creatine amidinohydrolase with the second concentration of [the substrate] creatine, wherein the second concentration of [the substrate] creatine is less than the first concentration of [the substrate] creatine,

(C) calculating a ratio of the second activity of the creatine amidinohydrolase encoded by the source nucleic acid sequence divided by the first activity of the creatine amidinohydrolase encoded by the source nucleic acid sequence,

(D) calculating a ratio of the second activity of the mutant creatine amidinohydrolase divided by the first activity of the mutant creatine amidinohydrolase,

(E) comparing the ratio calculated in step (iii)(C) to the ratio calculated in step (iii)(D), wherein a mutant creatine amidinohydrolase that has a reduced Km value as compared to the Km value of creatine amidinohydrolase encoded by the source nucleic

acid sequence has a greater ratio than the ratio for creatine amidinohydrolase encoded by the source nucleic acid,

(iv) selecting and isolating a desired mutant nucleic acid sequence that encodes a creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O$ → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM,

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectic point: about 4.5

Optimum temperature: about 40-50 °C (at pH of about 7.5)

Optimum pH: about 8.0-9.0 (at a temperature of about 37 °C)

(v) expressing the desired mutant nucleic acid sequence in a host to produce creatine amidinohydrolase, and

(vi) harvesting the produced creatine amidinohydrolase.

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PATENT
Attorney Docket No. 226749
Client Reference No. 20462 CPA-RI-Div

**SUPPLEMENTAL DECLARATION FOR REISSUE PATENT APPLICATION TO CORRECT "ERRORS" STATEMENT (37 C.F.R. 1.175)**

Patent Application No. 10/807,228

Applicant: Sogabe et al.

Filed: March 23, 2004

TC/AU: 1652

Examiner: E. Slobodyansky

Docket No.: 226749-Tak (Client Reference No. 20462-CPA-RI-RCE-DIV)

Customer No.: 23460

As a below named inventors, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name. I believe I am an original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**CREATINE AMIDINOHYDROLASE, PRODUCTION THEREOF AND USE THEREOF**

I previously executed a Combined Declaration and Power of Attorney, submitted March 23, 2004, with respect to the subject application. I hereby reaffirm the statements made in the previously executed Combined Declaration and Power of Attorney.

In the previously executed Combined Declaration and Power of Attorney, I stated that I believed the original parent, U.S. Patent No. 6,080,553, to be partially inoperative by reason of claiming less than the applicants had a right to claim. I continue to have such a belief for the reasons stated in the previously executed Combined Declaration and Power of Attorney and this Supplemental Declaration for Reissue Patent Application.

The original patent describes and claims a creatine amidinohydrolase, reagent, method of production thereof, and method of use thereof. The creatine amidinohydrolase is defined by physicochemical properties, such as action, optimum temperature, optimum pH, Km value, molecular weight, and isoelectric point. The isoelectric point (pI) for the creatine amidinohydrolase is recited in the specification and claims of the original patent as 3.5, but in fact was and is 4.5. Additionally, we have identified a typographical error in the nucleotide sequence of SEQ ID NO: 2. Specifically, nucleotide residue 435 of SEQ ID NO: 2 is recited as cysteine (C), but in fact was and is guanine (G). These errors and any other errors (i.e., all errors) were made without any deceptive intent on the part of the applicants and were discovered only relatively recently. To rectify these and other errors (such as the failure to claim a method of preparing a creatine amidinohydrolase as recited in the new claims added by way of the present reissue application), the specification and claims of the original patent have been amended by way of specification, claim, and abstract changes previously filed in the present reissue application as well as in U.S. Patent Application No. 09/940,941 (U.S. Patent No. RE38687), each of which is a divisional reissue of the original patent. Every error in the original patent which was corrected in the present reissue application, and is not covered by a prior oath/declaration submitted in this reissue application, arose without any deceptive intention on the part of the applicants.

I declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

In re Appln. of Sogabe et al.
Application No. 10/807,228

Full name of sole or first inventor: ATSUSHI SOGABE
Inventor's signature Atsushi Sogabe
Date Sep. 30, 2005 Country of Citizenship: Japan
Residence: Tsuruga-shi, Fukui, Japan
*(city/state or country)*
Post Office Address: c/o Toyo Boseki Kabushiki Kaisha, Tsuruga Institute of Biotechnology, 10-24, Toyo-cho, Tsuruga-shi, Fukui 914 Japan
*(complete mailing address)*

Full name of second joint inventor, if any: TAKASHI HATTORI
Inventor's signature TAKASHI HATTORI
Date Sep. 27. 2005 Country of Citizenship: Japan
Residence: Tsuruga-shi, Fukui, Japan
*(city/state or country)*
Post Office Address: c/o Toyo Boseki Kabushiki Kaisha, Tsuruga Institute of Biotechnology, 10-24, Toyo-cho, Tsuruga-shi, Fukui 914 Japan
*(complete mailing address)*

Full name of third joint inventor, if any: YOSHIAKI NISHIYA
Inventor's signature Yoshiaki Nishiya
Date Sep. 27. 2005 Country of Citizenship: Japan
Residence: Tsuruga-shi, Fukui, Japan
*(city/state or country)*
Post Office Address: c/o Toyo Boseki Kabushiki Kaisha, Tsuruga Institute of Biotechnology, 10-24, Toyo-cho, Tsuruga-shi, Fukui 914 Japan
*(complete mailing address)*

Full name of fourth joint inventor, if any: YOSHIHISA KAWAMURA
Inventor's signature Yoshihisa Kawamura
Date Sep. 22. 2005 Country of Citizenship: Japan
Residence: Tsuruga-shi, Fukui, Japan
*(city/state or country)*
Post Office Address: c/o Toyo Boseki Kabushiki Kaisha, Tsuruga Institute of Biotechnology, 10-24, Toyo-cho, Tsuruga-shi, Fukui 914 Japan
*(complete mailing address)*